Original Article

Sensitivity to Antibiotics of Bacteria Exposed to Gamma Radiation Emitted from Hot Soils of the High Background Radiation Areas of Ramsar, Northern Iran

Seyed Mohammad Javad Mortazavi[1,2], Samira Zarei[3], Mohammad Taheri[4], Saeed Tajbakhsh[3,5], Seyed Alireza Mortazavi[2], Sahar Ranjbar[6], Fatemeh Momeni[6], Samaneh Masoomi[6], Leila Ansari[6], Mohammad Mehdi Movahedi[7], Shahram Taeb[2], Sina Zarei[8], Masood Haghani[2]

[1]Department of Diagnostic Imaging, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111, USA

[2]Ionizing and Non-ionizing Radiation Protection Research Center (INIRPRC), Shiraz University of Medical Sciences, Shiraz, Iran

[3]Department of Microbiology and Parasitology, Faculty of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran

[4]Department of Microbiology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

[5]The Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Sciences, Bushehr, Iran

Correspondence to SMJ Mortazavi, PhD, Professor of Medical Physics, Department of Diagnostic Imaging, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111, USA

E-mail: mmortazavi@sums.ac.ir

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Abstract

Background: Over the past several years our laboratories have investigated different aspects of the challenging issue of the alterations in bacterial susceptibility to antibiotics induced by physical stresses.

Objective: To explore the bacterial susceptibility to antibiotics in samples of *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S. typhimurium*), *Staphylococcus aureus*, and *Klebsiella pneumoniae* after exposure to gamma radiation emitted from the soil samples taken from the high background radiation areas of Ramsar, northern Iran.

Methods: Standard Kirby-Bauer test, which evaluates the size of the zone of inhibition as an indicator of the susceptibility of different bacteria to antibiotics, was used in this study.

Results: The maximum alteration of the diameter of inhibition zone was found for *K. pneumoniae* when tested for ciprofloxacin. In this case, the mean diameter of no growth zone in non-irradiated control samples of *K. pneumoniae* was 20.3 (SD 0.6) mm; it was 14.7 (SD 0.6) mm in irradiated samples. On the other hand, the minimum changes in the diameter of inhibition zone were found for *S. typhimurium* and *S. aureus* when these bacteria were tested for nitrofurantoin and cephalexin, respectively.

Conclusion: Gamma rays were capable of making significant alterations in bacterial susceptibility to antibiotics. It can be hypothesized that high levels of natural background radiation can induce adaptive phenomena that help microorganisms better cope with lethal effects of antibiotics.

Keywords: Microbiology; Background radiation; Microbial sensitivity tests; Electromagnetic radiation; Gamma rays; Hormesis

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[6]Medical Physics Department, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[7]Medical Physics and Medical Engineering Department, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[8]Varastegan University of Medical Sciences, Shiraz, Iran

Introduction

The extraordinary levels of natural radioactivity reported in the high background radiation areas of Ramsar, northern Iran, may be attributed to high concentrations of ^{226}Ra and its decay products, which have been brought to the surface by water of hot springs. The genus *Salmonella* is an important member of the family Enterobacteriaceae. *Salmonella* organisms are short Gram-negative facultative anaerobic rods that cause serious infections in humans and animals.[1,2]

Ramsar is a coastal city in northern Iran with some areas known to have the highest levels of natural background radiation in the world. The mean annual dose for the residents of high background radiation areas (HBRAs) of Ramsar is about 10 mSv,[3] and a very small proportion of the residents receive doses as large as 260 mSv/y. As the accepted dose limit for radiation workers in Iran is 20 mSv/y, residents of the aforementioned areas receive a much higher annual dose than the permissible limit of occupational exposure.[4] Although it is not well known, it is widely believed that the high background radiation in the HBRAs of Ramsar is primarily due to the presence of very high concentrations of ^{226}Ra and its decay products.

At far distances from Ramsar, igneous bedrocks at high-altitude areas have high concentrations of ^{238}U. Uranium is insoluble in anoxic ground water but it decays into ^{226}Ra, and radium dissolves in ground water. Dissolved radium is carried by underground streams to the surface. At hot spring, where underground water reaches the surface, calcium carbonate precipitates out of solution and ^{226}Ra substitutes for calcium forming $RaCO_3$. This is why high concentrations of $RaCO_3$ can be found in the residue of hot springs. Interestingly, Ra-enriched rock from the hot springs has been used by some local people as building materials to construct their houses.[4]

There are also hot springs with different concentrations of radioactivity in the HBRAs of Ramsar. Both local inhabitants and tourists use these hot springs as health spas. No increased incidence rates of cancer or leukemia in the residents of HBRAs of Ramsar has been reported so far. There is also no difference between the life span of the residents of HBRAs and those of a nearby area with normal levels of background radiation.[5] Over the past years, our laboratory has studied different aspects of the challenging issue of the health effects of human exposure to highly elevated levels of natural radiation in Ramsar.

According to WHO, microbial resistance has threatened the effective prevention and treatment of infections. WHO believes that microbial resistance can be considered a serious threat to human health all around the globe. It has been reported that microbial resistance has led to large financial health care burden for patients with resistant infections due to longer duration of disease, more tests and administration of more expensive drugs.[6] This study was aimed at investigating bacterial susceptibility to antibiotics in control and irradiated samples of *S. enterica* subsp. *enterica* serovar Typhimurium (*S. typhimurium*), *S. aureus*, and *K. pneumoniae*.

Materials and Methods

Soil Samples

Environmental monitoring in *Talesh Mahalleh* was performed using a RDS-110 (RADOS. Inc., Finland) multi-purpose survey meter. Before soil sampling, absorbed dose rates in air were measured at ground level and one meter above the ground level. Previously, in another study, soil samples were sent to the National Radiation Protection Department (NRPD) of the Iranian Nuclear Regulatory Authority for gamma spectroscopy. The concentrations of ^{226}Ra, ^{232}Th and ^{40}K radionuclides in each soil sample were measured by a high purity germanium (HPGe) gamma ray spectrometer (Canberra Industries Inc.).

Antimicrobial Susceptibility Tests

The bacterial strains used in this study (*K. pneumoniae* PTCC 1290, *S. typhimurium* 1709, *S. aureus* 29213) were obtained from the Iranian Research Organization for Science and Technology (IROST). The samples were cultured on nutrient agar. Salmonella-Shigella (SS) agar culture media were used for the isolation of microorganisms. The culture plates were incubated at 35 °C for 24 hrs.

Antimicrobial susceptibility test of bacterial strains was performed using disc diffusion method (Kirby-Bauer) on Müller-Hinton agar plates. The fresh cultures were diluted in TSB (Tryptone-based Soy) broth and matched with the 0.5 McFarland turbidity standards to get 1.5×10^8 CFU/mL as total count.

Bacterial suspensions were spread on Müller-Hinton agar (Lio, Italy) plates. The antibiotic discs were placed on the surface of the plates and they were then incubated at 35 °C for 24 hrs. The inhibition zone around each antibiotic disc was measured.

TAKE-HOME MESSAGE

- Microbial resistance is an increasing problem worldwide.
- Exposure to ionizing and non-ionizing radiation may affect microbial susceptibility to antibiotics.
- Ramsar is a region with high background radiations in northern Iran.
- Exposure to gamma radiation emitted from hot soil taken from areas with high background radiation cause significant alterations in bacterial susceptibility to antibiotics.
- This effect is particularly significant in *K. pneumoniae*.

Drug susceptibility test was performed for trimethoprim-sulfamethoxazole (SXT 1/25 + 23/75 µg), nitrofurantoin (FM 300 µg), vancomycin (V 30 µg), cephalexin (CN 30 µg), tetracycline (TE 30 µg), cephalothin (CF 30 µg), nalidixic acid (NA 30 µg), ciprofloxacin (CP 5 µg), and gentamicin (GM 10 µg). All culture media and antibiotic discs were purchased from HiMedia Company.

Results of antibiotic susceptibility assay before and after exposure to Ramsar soil were recorded and analyzed. The inhibition zone of each plate was recorded. Three replicate agar plates were used, according to the Clinical and Laboratory Standard Institute (CLSI) 2013 guidelines.

Irradiation of Samples and Incubation

Three culture plates for each microorganism were placed on a box filled with Ramsar hot soil (soil with high concentration of radionuclides such as ^{226}Ra). The exposure rate was measured by a Fluke 451 ion chamber survey meter. Three non-exposed plates were also used for each microorganism as controls. At the surface of the culture medium, the exposure rate was 0.38 mR/h. After incubating the bacteria and allowing them to grow at 37 °C for 18 hrs, the diameter of the clear bacteria-free zone appeared around each antibiotic disk was measured.

Results

The mean diameters of the inhibition zones of non-irradiated control samples of *S. typhimurium*, *S. aureus*, and *K. pneumoniae* were compared to those of samples irradiated with gamma radiations emitted from Ramsar hot soil (Table 1).

The maximum alteration of the diameter of the inhibition zone was found in *K. pneumoniae* when it was tested with ciprofloxacin. The mean diameter of no-growth zone in non-irradiated control samples of

K. pneumoniae was 20.3 (SD 0.6) mm; it was 14.7 (SD 0.6) mm in irradiated samples. On the other hand, the minimum changes in the diameter of the inhibition zone were found in *S. typhimurium* and *S. aureus* when they were tested with nitrofurantoin and cephalexin, respectively (Table 1).

Discussion

We found that exposure to gamma rays caused significant alterations in bacterial susceptibility to antibiotics. The effect was particularly significant in *K. pneumoniae*. Substantial evidence indicates that many bacteria are becoming resistant to most or even all of the current available antibiotics.[7,8] Investigation of the effects of electromagnetic radiation on bacteria may thus play a key role in exploring the possibility of controlling the increasing microbial resistance. To the best of our knowledge, this is the first study investigating the effects of exposure of microorganisms to above-the-normal levels of natural radiation.

Studies performed on both ionizing and non-ionizing electromagnetic radiation support our findings. Our findings are in line with those of Farrag who showed significant differences between the means of antibiotic sensitivity of *Pseudomonas aeruginosa* before and after exposure to 2000 cGys gamma rays.[9]

Moving to non-ionizing radiations, generally speaking, these findings are in line with those reported by Torgomyan and Trchounian in 2013 who showed that exposure of bacteria to non-ionizing electromagnetic fields can lead to changes in their sensitivity to different chemicals, including antibiotics.[10] They also concluded that exposure to electromagnetic fields may affect the cell-to-cell interactions in bacteria, and that bacteria might interact with each other through the electromagnetic fields in a sub-extremely high-frequency range.[10]

Table 1: Bacterial susceptibility to antibiotics in the control and irradiated samples. Values are mean (SD) of three measurements.

Bacteria	Antibiotics*	Diameter of no-growth zone (mm) Control	Irradiated	Δdiameter (Irrad – Cont)
S. typhimurium (PTCC 1709)	TE	13.5 (2.7)	12.3 (0.6)	-1.2
	V	15.3 (0.6)	14.3 (0.6)	-1.0
	CP	22.7 (0.6)	19.7 (1.2)	-3.0
	FM	20.7 (0.6)	21.0 (1.0)	+0.3
	NA	14.7 (0.6)	16.3 (0.6)	+1.7
S. aureus (PTCC 29213)	CN	15.3 (0.6)	14.7 (0.6)	+0.3
	TE	19.7 (0.6)	20.0 (0.0)	-1.0
	CF	15.3 (1.5)	14.3 (1.6)	-0.7
	GM	12.3 (0.6)	11.7 (0.6)	-1.0
	CP	21.7 (1.5)	20.7 (0.6)	-1.0
	FM	17.7 (2.1)	16.7 (0.6)	-1.2
	SXT	22.7 (1.5	20.0 (0.0)	-2.7
	NA	21.0 (1.0)	19.3 (1.2)	-1.7
K. pneumoniae (PTCC 1290)	GM	13.3 (0.6)	11.7 (0.6)	-1.7
	CP	20.3 (0.6)	14.7 (0.6)	-5.7
	FM	14.0 (1.7)	10.0 (0.0)	-4.0
	SXT	20.7 (1.2)	17.7 (0.6)	-3.0
	NA	20.7 (0.6)	17.3 (0.6)	-3.3

*CN Cephalexin, TE Tetracycline, CF Cephalothin, GM Gentamicin, CP Ciprofloxacin, FM Nitrofurantoin, SXT Trimethoprim-Sulfamethoxazol, NA Nalidixic acid, V Vancomycin

Furthermore, our findings are in line with the results obtained on the growth rate and antibiotic sensitivity of *Escherichia coli* after exposure to non-ionizing extremely low-frequency electromagnetic fields. It was found that exposure to extremely low-frequency electromagnetic fields (2 mT, 50 Hz) significantly affects the growth rate of *E. coli* and *P. aeruginosa*.[11]

Findings of our present study were also in line with our previous experiments on *K. pneumoniae*. We have recently shown that *K. pneumoniae* exposed to 2.4 GHz Wi-Fi radiofrequency radiation for 3, 4.5, and 8 hrs exhibit significant variations in their susceptibility to antibiotics.[12] In our experiments, regardless of the type of antibiotics, the mean diameters of the inhibition zones after three hours of exposure were less than those exposed for 4.5 hours. Interestingly, following this rise, a fall was observed in the sensitivity to antibiotics in the bacteria exposed for eight hours.[12]

Furthermore, we have previously reported that the observed decrease in diameter of the zone of inhibition in *K. pneumoniae* after exposure to mechanical ultrasound waves, can be explained as the induction of adaptive response.[13]

Although we do not know the mechanisms involved yet, we believe that the alterations observed in bacteria after exposure to gamma rays emitted from the hot soil samples of Ramsar can be due to changes in efflux pumps. Efflux pumps are transport proteins found in both Gram-positive and Gram-negative bacteria. It is widely accepted that efflux pumps are involved in removing toxic substances including antimicrobial agents from the intracellular space.[14] Further experiments are needed to clarify the possible role of efflux pumps in these radiation-induced alterations in bacterial susceptibility to antibiotics.

In conclusion, our findings can support the concept that high levels of background radiation can induce adaptive phenomena, which help microorganisms better cope with lethal effects of antibiotics.

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Conflicts of Interest: None Declared.

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